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EXAMINER	
FITZGERALD, D	

18N2/0607

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ART UNIT	PAPER NUMBER
1812	13

DATE MAILED: 06/07/94

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), ~~_____ days~~ from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-32 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 1-32 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).

12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____ ; filed on _____

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION

1. The receipt of the preliminary amendments filed 19 July 1993, 10 January 1994, and 18 March 1993, all in connection with the submission of a computer-readable Sequence Listing, is acknowledged. The Sequence Listing *per se* satisfies the requirements therefor.

2. Applicant is advised that the Examiner has rectified the following informality or informalities in the specification: page 7, line 11, change "2A to 2D" to "2A, 2B, 2C, and 2D".

It is also brought to Applicant's attention that the Examiner has corrected the citation of reference No. 8 on the form PTO-1449.

3. Claims 16-18 and 25-28 are objected to under 37 C.F.R. § 1.821(d) for failing to recite sequence identifiers (*i.e.*, SEQ ID NO's) concurrently with the recited sequences.

4. Claims 1 and 22 are objected to as being duplicate claims. One of the duplicate claims should be cancelled; alternatively, the objection will be obviated if the term "optionally" is deleted from claim 22. See 37 C.F.R. § 1.75(b); Applicant's attention is also directed to M.P.E.P. § 706.03(k).

5. The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make and/or use the invention, *i.e.*, failing to provide an enabling disclosure.

The specification does not provide enabling support commensurate in scope with the breadth of prorelaxins encompassed by the claims. A "non-naturally occurring C chain" encompasses peptide linkers as short as a single amino acid and as long as hundreds or thousands of residues. Critical to the usefulness of the prorelaxin proteins, however, is that they be able to properly fold and form the A chain-B chain disulfide linkages characteristic of mature relaxins. One of ordinary skill in the art would expect a prorelaxin having a very short C chain to be nonfunctional because the protein would simply not be long enough to fold back upon itself so as to place the cross-linking Cys residues in apposition. For a very long C chain, on the other hand, the artisan would expect the entropic contribution of the linking peptide to make the energetics of correct refolding unfavorable. The artisan would therefore not expect to be able

to use prorelaxins having arbitrarily long or arbitrarily short C peptides according to the invention. The only guidance in the specification relative to this point is the teaching that "C-chains having about 8 to 15 amino acids" are preferred embodiments; the issue of minimum and maximum operable lengths is not addressed. In the absence of suitable guidance, it would require undue experimentation of the artisan to determine the full range of operative embodiments encompassed by the claims.

The specification furthermore does not provide the guidance necessary to permit the ordinarily skilled artisan to practice an arbitrarily chosen embodiment of the invention. As noted above, the invention encompasses a broad range of prorelaxin proteins. Applicant's disclosure, however, provides only a single protocol for refolding the "mini-C" prorelaxins of the invention (pages 31-32), *i.e.*, those having SEQ ID NO: 3 as the C peptide (Fig. 1). No guidance whatsoever is provided to direct the artisan to refolding protocols which will be useful to prepare prorelaxins other than the "mini-C". Furthermore, as Applicant acknowledges (page 19, line 20):

> For refolding proteins, such as prorelaxin, that contain cysteine residues and in the naturally occurring form contain disulfide bonds, the reduction/oxidation conditions present in the solubilization and refolding steps are critical and protein specific.

One skilled in the art of the refolding of recombinantly produced proteins would find this statement accurate. Thus since the redox conditions employed in the solubilization and refolding of recombinantly produced prorelaxins are protein-specific and critical to the practice of the invention, this example, even though explicit and detailed in its prescriptions, does not teach how to practice the full scope of the invention.

The specification does not support the use of carboxypeptidase B to excise a C peptide from a prorelaxin protein. As the name implies, carboxypeptidases cleave C-terminal amino acid residues from a peptide chain; they are generally recognized not to be capable of cleaving an internal peptide bond. See, for example, Lehninger, *Biochemistry* (2^{d} ed., 1975), page 560.

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6. Claims 1-32 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

7. Claims 1-8, 13-22, and 25-29 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 and the rejected claims dependent therefrom are incomplete and indefinite for failing to recite a refolding step, which is requisite to the practice of the method as disclosed.

Claim 5 is ambiguous in that it could be read to encompass the use of {[trypsin] or [Arg-C + CPB]}, or, alternatively, {[trypsin or Arg-C] + CPB}; the two interpretations are of different scope.

Claims 16-20 and 25-29 are indefinite with respect to the recitation of "is comprised of"; it is not clear whether the intended meaning is "consists of" or "comprises". The claims should be amended to recite one of the latter alternatives.

Claim 21 employs an improper Markush formulation inasmuch as the method "comprises" the various alternative embodiments. See M.P.E.P. § 706.03(y). This ground of rejection will be obviated if the claim is amended to recite "a process which includes a step selected from the group consisting of".

Claim 22 recites "formulation buffer" without antecedent basis in the specification. The examiner suggests deleting the word "formulation" in favor of, *e.g.*, "a suitable".

8. The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

9. Claims 1-8, 21-24, and 30-32 are rejected under 35 U.S.C. § 103 as being unpatentable over Hudson *et al.* (U.S. 5,179,195) in view of Chang *et al.* (*BBRC* 171: 818-26, 1990) and *Enzyme Nomenclature* ("*EN*", IUB, 1984).

Hudson discloses DNA encoding human H2 preprorelaxin, *i.e.*, the naturally occurring signal-B-C-A polypeptide (abstract; Figs. 2A-2E), and methods of using the DNA to produce mature H2 relaxin in heterologous expression systems (cols. 6-7). *E. coli* is disclosed as an exemplary preferred host cell (col. 6, lines 53-55). The reference teaches the desirability of modifying the C chain of prorelaxin to facilitate its excision during subsequent processing (col. 7, lines 34-44) and claims an embodiment having a modified (*i.e.*, non-naturally occurring) C chain (claim 4). The reference does not exemplify a *particular* embodiment of a pro- or prepro-relaxin having a modified C chain, DNA encoding it, or its expression in *E. coli*; neither does it teach the enzymes which would advantageously be employed to process such a prorelaxin. The reference does not teach cyclization of the N-terminal Gln of the A chain in the mature product.

Chang and *Enzyme Nomenclature* are relied upon as they provide evidence that a variety of peptidases having known cleavage patterns were available in the art. In particular, Chang discloses Lys C (cleaving K↓X), Arg C (R↓X), and Asp N (X↓D) (abstract), and *EN* describes trypsin ([R,K]↓X) and carboxypeptidase B (X↓[K,R]).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the sequence of the H2 preprorelaxin disclosed by Hudson, using molecular genetic techniques as were routinely employed in the art, such that the B/C and C/A peptide junctions were engineered to have sequences corresponding to the preferential cleavage sites of readily available endopeptidases, such as those disclosed by Chang and the *EN* publication, because Hudson teaches that the C peptide can be so desirably modified in order to facilitate its excision in the course of converting recombinantly produced prorelaxin to mature relaxin. It would further have been obvious to express the modified prorelaxin in a suitable host cell and to cleave it with peptidases specific for the engineered cleavage sites in order to obtain mature relaxin, according to Hudson. To purify the protein using conventional techniques and to formulate the product in a suitable pharmaceutical carrier (buffer) would have been *prima facie* obvious since the purpose of making relaxin is to use it in pharmaceutical applications. The claimed invention would have been *prima facie* obvious as a whole at the time it was made, especially in the absence of evidence to the contrary.

10. Claims 14 and 15 are rejected under 35 U.S.C. § 103 as being unpatentable over Hudson, Chang, and *Enzyme Nomenclature* as applied to claims 1-8, 21-24, and 30-32 above,

and further in view of Stults *et al.* (*Biomed. Environ. Mass Spectrom.* 19: 655-64, 1990) and Dimarchi *et al.* (*Int. J. Pept. Prot. Res.* 19: 88-93, 1982).

Stults teaches that native human relaxin bears a pyroglutamic acid (cyclized glutamine) residue at the N-terminus of the A chain (abstract).

Dimarchi teaches that N-terminal Gln residues spontaneously cyclize, particularly in the presence of weak acids (abstract).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to treat the relaxin generated from a modified prorelaxin (as was obvious over Hudson, Chang, and *EN*) with weak acids in order to convert the N-terminal Gln of the A chain to pyroglutamic acid, according to Dimarchi, because Stults teaches that the naturally-occurring relaxin hormone bears this modification. It would have been obvious to the artisan that heat would accelerate the reaction because the artisan would have expected the system to exhibit Arrhenius kinetics. The claimed invention would have been *prima facie* obvious as a whole at the time it was made, especially in the absence of evidence to the contrary.

11. Claims 9-13 are rejected under 35 U.S.C. § 103 as being unpatentable over Hudson, Chang, and *Enzyme Nomenclature* as applied to claims 1-8, 21-24, and 30-32 above, and further in view of Olson (U.S. 4,518,526).

Olson provides evidence that the manipulations required to obtain active heterologously expressed proteins from eukaryotic host cells were known to those of skill in the art; the reference particularly considers recovery of proteins present in inclusion bodies. The reference teaches in detail the considerations which must be addressed in designing a successful recovery protocol (cols. 9-21), including the use of denaturants to solubilize protein (Example E), buffers which will maintain solubility (Example G), and methods to properly refold the purified protein (Example H). Several specific protocols are exemplified.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to solubilize and renature a prorelaxin designed after the teachings of Hudson, Chang, and *EN,* incorporating in the purification and refolding protocol such solubilization and renaturation method steps as were known in the art, as is evidenced by Olson, because Olson teaches that such steps permit the recovery of active proteins in good yields. The claimed invention would

have been *prima facie* obvious as a whole at the time it was made, especially in the absence of evidence to the contrary.

12. Claims 16-20 and 25-29 are free of the prior art. The C-peptides of these embodiments are substantially shorter than that of the naturally occurring prohormone (see, *e.g.*, Hudson, *supra*), and for substantially the reasons discussed above in the objection under § 112, first paragraph, one would not have expected *a priori* that these proteins would be capable of folding correctly to yield mature human relaxins. The art of record furthermore provides no motivation to substantially shorten the C peptide of prorelaxin.

No claim is allowed.

13. The art cited but not relied upon is considered pertinent to Applicant's disclosure. Hudson *et al.* (U.S. 5,145,962) discloses and claims human H1 preprorelaxins. Proteins having an altered C chain are claimed (claim 4).

14. Any inquiry concerning this communication should be directed to David Fitzgerald at telephone number (703) 308-3934, fax numbers (703) 305-3014 or 308-4227, or through the Group 1800 receptionist at (703) 308-0196. Note also that papers of record may be submitted to Group 1800 by fax at the numbers above; refer to 1096 OG 30.

David L. Fitzgerald
Examiner
25 May 1994

GARNETTE D. DRAPER
PRIMARY EXAMINER
ART UNIT 1812